—Mini Review—

Transgenesis Via Intracytoplasmic Sperm Injection (ICSI) in Rodents

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Abstract: Intracytoplasmic sperm injection (ICSI) has been successfully achieved in mice and rats using a piezo-driven injection pipette. More than 30% of ICSI oocytes are capable of developing to full-term when the isolated sperm heads are microinjected. The ICSI technique has been applied not only to rescue infertile male strains, but also to produce transgenic rodents. ICSI-mediated DNA transfer, which mixes sperm heads and exogenous DNA solution and co-injects them into ooplasm, was as effective as conventional pronuclear DNA microinjection. The production efficiency of transgenic founders by ICSI-mediated DNA transfer was comparable between mice and rats, while the optimal DNA concentration for 1-min exposure was lower in rats than in mice. The production efficiency was improved when the membrane structure of sperm heads was partially disrupted by detergent or ultrasonic treatment before exposure to the exogenous DNA solution. Exogenous DNAs with various chain lengths have been stably integrated into rodent genomes of various genetic backgrounds using this method. ICSI-mediated DNA transfer in which preparation of pronuclear-stage fertilized zygotes is not required was not required would be alternative to conventional pronuclear DNA microinjection.

Key words: DNA transfer, ICSI-tg, Transgenic mouse, Transgenic rat

Introduction

Transgenic technology in mammals is increasingly important in the design and implementation of biological and physiological studies. Pronuclear microinjection of exogenous DNA is the most convenient and reproducible technique for production of transgenic animals, and the efficiency of producing transgenic mice and rats using this technique is generally 1 to 5% of the total injected zygotes or 10% of the newborn offspring. Sperm-mediated DNA transfer into mouse genomes was first reported by Lavitrano et al. [1]. They demonstrated that oocytes fertilized in vitro with exogenous DNA-bound sperm cells could develop into transgenic mice, with a maximum efficiency of 30% of 250 newborn offspring. This approach to producing transgenic mice appeared to be more effective and less laborious, but numerous numbers of experiments by other laboratories failed to reproduce their results [2]. Perry et al. [3] reported that transgenic mice were successfully produced by intracytoplasmic sperm injection (ICSI) of mouse oocytes using sperm heads co-incubated with exogenous DNA solution. ICSI-mediated DNA transfer has been found to be a reproducible technique in mice [4, 5] and rats [6, 7]. In addition to transgenesis via pronuclear microinjection, in vitro fertilization, and microinsemination, there is also testis-mediated DNA transfer, including direct delivery of exogenous DNA into the testis (see the review by Sato [8]). In the present manuscript, rodent transgenesis via ICSI using sperm cells as vectors of exogenous DNA is reviewed.

ICSI Protocols in Mice and Rats

ICSI has been used as a research tool for studying fundamental aspects of gamete interaction during fertilization and has been routinely applied to clinical treatment of male infertility in humans. Since 1988, normal live offspring following ICSI have been reported in several mammalian species, including small rodents, large domestic animals, and primates [9]. The first successful ICSI in rodents was achieved by Kimura and Yanagimachi (1995) [10] using a piezo-driven injection...
pipette 5 µm in diameter to dissociate the sperm head from the tail and to drill the zona pellucida and oolemma of mouse oocytes. When the mouse sperm head was aspirated into the injection pipette and microinjected into an oocyte, approximately 60% of the surviving oocytes were capable of developing into newborn mice.

An earlier attempt at piezo-ICSI in rats [11] suggested that microinjection of larger rat sperm heads is extremely difficult even though the use of piezo-driven versus the conventional pipette (7 to 11 µm in diameter) significantly improved the post-injection survival of rat oocytes (71 vs. 24%). We reported in 2002 that technical improvement to minimize the amount of medium injected with sperm heads makes it possible to produce rat offspring from ICSI oocytes [12]. Rat sperm heads were isolated by ultrasonic treatment and cryopreserved until use. When the sperm heads were held on (rather than aspirated into) the tip of much finer injection pipettes (2 to 4 µm in diameter) and expelled into oocytes, more than 30% of the ICSI oocytes were capable of developing into newborn rats (from our latest results). The volume of polyvinylpyrrolidone (PVP) brought into the oocytes by the improved method is also obviously smaller than by the conventional method because the improved method only releases the sperm heads from the pipettes prior to zona drilling and they are then taken up again for ICSI. In cattle, reducing the PVP concentration in the sperm-surrounding medium has been reported to increase the rate of pronucleus formation following ICSI [13].

Transgenesis Via ICSI

In 1999, Perry et al. [3] reported the first successful production of transgenic mice by ICSI using sperm heads co-incubated with exogenous EGFP DNA solution. ICSI-mediated DNA transfer provided similar efficiencies for production of transgenic rodents (3.8 to 4.5% of the transferred embryos for mice [3], 0.9 to 8.2% of the transferred embryos for rats [7]) when compared to the conventional pronuclear DNA microinjection. The standard protocol, as shown in Fig. 1, is as follows: (1) isolation of sperm heads and partial disruption of acrosomal/plasma membranes; (2) co-incubation of the sperm heads with exogenous DNA solution; (3) piezo-ICSI, followed by temporal in vitro culture and embryo transfer to foster mothers; and (4) identification of transgenes in the newborn offspring.

Disruption of the spermatozoal membrane would facilitate the binding of exogenous DNA molecules to sperm heads. Francolini et al. [14] reported that exogenous DNA could reversibly bind to the subacrosomal segment of mouse spermatozoa. Different procedures have been used to induce
To improve Production Efficiency

Low integration of exogenous DNA is still an obstacle to the widening of the transgenic technology. It was reported in 2003 that recombinase-A protein (RecA)-coated ssDNA was more likely to be integrated into goat and pig genomes after pronuclear DNA microinjection (6- and 10-fold higher than controls, respectively) [19]. In mice, the production efficiency of transgenic founders was improved only if the exogenous DNA was previously coated with the RecA and then subjected to ICSI-mediated transgenesis [5]. ICSI-mediated DNA transfer using RecA-coated EGFP ssDNA (5 to 40 µg/ml) produced transgenic mice at efficiencies of 3.6 to 11.1% of the transferred embryos, while the same protocol using non-coated ssDNA resulted in no transgenic offspring. However, we found that the RecA-coating of the exogenous EGFP and OAMB DNAs contributed very little to the production of transgenic rats by both pronuclear DNA microinjection and ICSI-
mediated DNA transfer [20].

Very recently, Suganuma et al. [21] indicated that hyperactive Tn5 mutant transposase assists the integration of exogenous DNA into both the inbred C57BL/6 mouse genome (4.3% of the transferred embryos) and hybrid B6D2F1 mouse genome (13.4% of the transferred embryos, vs. 1.1% in the transposase-free control group) when the transposase-DNA complexes are co-injected with spermatozoa. Since the positive effect of Tn5 transposase-mediated mouse transgenesis was exerted not only from ICSI but also from round spermatid injection (ROSI), such a pretreatment for exogenous DNA may be applicable for transgenesis using azoospermic animals.

Conclusion

ICSI-mediated DNA transfer is as effective as conventional pronuclear DNA microinjection in terms of the efficiency of producing transgenic rodents (up to several percent of the total number of treated oocytes), the stability of transgenes, and the applicable size of the exogenous DNA (up to a few hundred kbp). A possible advantage of applying ICSI for DNA transfer may have appeared in species (e.g. mastomys, Praomys coucha [22]) or strains of unique genetic characteristics (e.g. t\(^w5/t\(^w32\) mutant mouse [23]) from which recovery of pronuclear-stage fertilized zygotes after superovulation is difficult. In fact, practical application of ICSI-mediated DNA transfer using a growth hormone gene-deficient (Dwarf) rat strain reduced the cost of producing one founder to one-tenth when compared with that from pronuclear DNA microinjection. Since labor and space for animal care can also be significantly reduced, ICSI-mediated DNA transfer would become a possible alternative to the conventional pronuclear DNA microinjection under these circumstances.

References


